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Review

Clinical efficacy of carbocysteine in COPD: beyond the mucolytic action

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Abstract: Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease with a versatile and complicated profile, being the fourth most common single cause of death worldwide. Several research groups have been trying to identify possible therapeutic approaches to treat COPD, such as the use of mucoactive drugs which includes carbocysteine. However, their role in the treatment of patients suffering from COPD remains controversial due to COPD's multifaceted profile. In the present review, 72 articles, published in peer-reviewed journals with high impact factor, have been analyzed in order to provide significant insight and increase the knowledge about COPD considering the important contribution of carbocysteine in reducing exarcebations which occurs via multiple mechanisms. Carbocysteine is in fact able to modulate mucins and ciliary functions, counteract viral and bacterial infections as well as oxidative stress, offering cytoprotective actions. Furthermore, carbocysteine improves steroid responsiveness and exerts anti-inflammatory activity. This analysis demonstrates that the use of carbocysteine in COPD patients represents a well-tolerated treatment, with favorable safety prolife that might contribute to a better quality of life for patients suffering from this serious illness.

Keywords: Carbocysteine; COPD treatment; Mucolytics; Exacerbations

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1. Introduction

For the past 20 years, extensive research has been focusing on the pathogenesis and treatment of Chronic Obstructive Pulmonary Disease (COPD). Based on clinical presentation, genetic background, pathophysiology, and therapeutic response COPD is classified as a heterogeneous disease with a versatile and complicated profile. It is crucial to search for precision medicine to treat this pathology [1], which is estimated to be the fourth most common single cause of death worldwide [2] constituting a major public health problem [3].

According to several studies observed throughout the years, COPD pathogenesis has been associated with airway inflammation, oxidative stress, bacteria colonization, cilium-beating dysfunction, and mucus hypersecretion. In particular, a review from 2010 has stressed the importance of chronic mucus hypersecretion, the most typical symptom of

chronic bronchitis [4,5], which is present independently of airflow obstruction [6]. Moreover, due to its heterogeneity, it has been challenging to distinct precisely COPD subtypes to choose the most appropriate treatment for each patient. In the last ten years, this point has been often reiterated along with the message that COPD prevention, diagnosis, and treatment should be a long-term, comprehensive, persistent, and individualized program.

Although mucoactive drugs have been widely used in clinical practice for a long time and their efficacy has been extensively addressed in the scientific literature, their role in the treatment of patients suffering from COPD remains controversial. There are numerous available mucoactive medicines for prescription, however, it is important to select them properly according to different diseases [1].

A subtype of COPD, known as an acute exacerbation of COPD (AECOPD) is associated with clinical and functional decline in COPD patients. The AECOPD is defined as an acute worsening of respiratory symptoms and requires additional therapy. Indeed, it accelerates the loss of lung function and leads to decreased health-related quality of life, significant economic costs, and increased mortality rates [7].

Mucoactive agents have been used in a large range of pathologies due to their recognizable utility potential [8]. In 2006, Poole and Black [5] published a systematic review of 26 randomized, placebo-controlled studies which recruited more than 7300 patients with COPD. In this review, it was shown that treatment with mucolytics, for at least 2 months, significantly reduced the number of exacerbations. Since then, other reviews have been published in the Cochrane Library evaluating the effectiveness of mucolytic agents in the prevention of exacerbations [9]. The correlations found in the studies produced controversial insights and, none of them was able to verify the effect of mucolytics for a long period [10]. The evidence gathered was not precise enough to identify the target population for mucolytic/antioxidant agents in COPD as randomized control trials (RCTs) had different quality standards, the duration, and doses of treatment were different among the studies as well as the number of exacerbations in the year previous the enrolment [8,10]. As a result, the national guidelines for COPD management in Europe reported different recommendations. N-acetylcysteine/oral carbocysteine were both recommended in the Czech Republic, England and Wales (with caution), Poland, Russia, and Spain, but not recommended in Finland, France, and Portugal [11].

The 2017 version of Global Initiative for Chronic Obstructive Lung Disease (GOLD) [12] presented a table entitled "Anti-inflammatory therapy in stable COPD". This table stated that regular treatment with mucolytic/antioxidant agents such as carbocysteine, N-acetylcysteine (NAC), and erdosteine modestly improved health status by reducing the risk of exacerbations in selected populations not receiving inhaled corticosteroids (ICS). This statement was classified as evidence B. According to the description of levels of evidence, sources of evidence B are RCTs with important limitations as these studies included only a limited number of patients, post hoc or subgroup analysis of RCTs, or meta-analyses of RCTs. Moreover, the body of evidence was limited because of important restrictions (methodological flaws, small numbers, short duration), undertaken in a population that differed from the recommended target population and the results were somehow inconsistent. The last version of GOLD released in 2020 [13] confirmed previous statements keeping the evidence to be still classified as B. Due to the heterogeneity of studied populations, treatment dosage, and concomitant treatments, currently available data do not allow the precise identification of potential target populations for mucoactive agents in COPD. Thus, GOLD recommends giving mucolytic and antioxidant agents (N-acetylcysteine, carbocysteine, and erdosteine) to treat COPD patients that are not receiving inhaled corticosteroid therapy. This suggestion could help to reduce COPD exacerbations and give a small improvement in health status to these patients.

Due to the heterogeneity detected in the studies available concerning COPD and possible mucoactive therapies, it has become a very controversial topic. Recently, a group of Chinese pulmonary physicians worked together to complete a consensus based on a literature review to define guidelines on COPD mucoactive treatment. The specialists in the

field concluded that, in general, mucoactive agents play an important role in COPD without any association to an increase in adverse effects. However, they repeated once again that precise treatments in the targeted COPD population need further investigation with stratification strategy [1]. Moreover, to this date, no head-to-head clinical studies on the same COPD patients across different mucoactive agents have been conducted to directly compare their efficacy profile. It is also important to take into consideration that the cellular and molecular mechanisms underlining the clinical efficacy of mucolytics are largely unknown.

The present review provides a comprehensive vision regarding the clinical efficacy of a mucoactive drug, carbocysteine, and insights into its cellular and molecular activities that could explain its clinical efficacy.

2. Materials and Methods

The EMBASE, Medline, and Cochrane review databases were used to conduct a systematic literature search. This search was also extended to grey literature sources, such as abstracts from the Congress of the European Respiratory Society (journal) or American Thoracic Society (ATS) Journal. Some of the keywords applied for the investigation were carbocysteine, COPD or Chronic Obstructive Lung Disease, chronic bronchitis, exacerbations, expectorants, mucolytics, and antioxidants. Throughout the exploration English-language scientific papers and literature reviews on molecular and cellular mechanisms of carbocysteine and clinical efficacy of carbocysteine in patients with COPD were also identified.

No time limit has been set for the search neither exclusion criteria. All reported molecular and clinical outcomes were evaluated with no restrictions on the type of publication or stage, sample type, or trial design (RCTs, as well as open, controlled, and uncontrolled trials, were included in the evaluation).

The initial literature search identified 363 published articles, 110 of which were potentially relevant references. The main topics were critically evaluated during the discussion and after the meeting, each expert carried out an independent qualitative systematic literature revision for the topic assigned. The references of the identified articles were reviewed for additional sources. Eventually, 80 references were eligible for this review after group discussion; disagreements were resolved by consensus and finally, 72 references were included in this review. Considering the nature of this work, for the sake of brevity, we do not list or provide specific comments on the exclusion criteria of papers that were judged less relevant or not strictly related by the panel of experts.

3. Molecular Effects of Carbocysteine

Carbocysteine (R-2-amino-3[(carboxymethyl)thiol] propionic acid) is a dibasic amino acid, biologically active. The carbocysteine molecule (Fig. 1) is characterized by the presence of a bound sulfydrilic group.

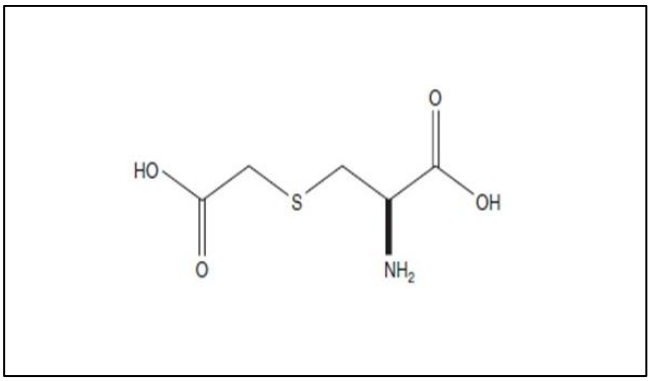

Figure 1. Chemical structure of carbocysteine.

The structure and mechanism of action of carbocysteine differ from other commonly available mucolytic drugs. For instance, N-acetylcysteine (NAC) and erdosteine bear free sulfhydryl (thiol) groups that allow them to split glycoprotein bonds in mucus [14]. Instead, a study using animal models has demonstrated that carbocysteine increases chloride transport across the airway epithelium, which may also contribute to its mucoregulatory action [15].

4. Pharmacokinetics

Carbocysteine is available in two forms for the oral preparation. It can be presented conjugated to its lysine salt (SCMC-Lys) or in its active form (SCMC), where the lysine group is cleaved on gastric absorption. This molecule is rapidly well absorbed after oral administration achieving its peak serum concentration after 1 to 2 hours (h) and its plasma half-life is 1h33minutes [16]. Carbocysteine appears to penetrate lung tissue and respiratory mucus, suggesting local action [17,18] and its kinetics fits a one-compartment open model [16].

Metabolism of carbocysteine is known to be especially complex, as several pathways such as decarboxylation, N-acetylation, sulfoxidation, deamination\transamination, and ester glucuronidation are involved at different degrees. Approximately 30% to 60% of the drug is excreted in urine without being metabolized [14,19].

Carbocysteine resets the balance between sialomucins and fucomucins by increasing sialomucins concentration and decreasing fucomucins levels. Besides, it enhances chloride transport across the airway epithelium. Therefore, initially, carbocysteine was introduced into the clinic as only a mucoregulator agent. However, a substantial number of studies carried out in recent years have better characterized the mechanism of action of carbocysteine, highlighting a series of useful effects in the management of COPD. These studies allow us to consider carbocysteine as a drug with several actions useful to a wide spectrum of acute and chronic diseases of the respiratory tract.

The contribution of carbocysteine in reducing exacerbations observed in COPD patients occurs via multiple mechanisms:

1) Modulating mucins and ciliary functions;
2) Counteracting viral infections;
3) Counteracting bacterial infections;
4) Counteracting oxidative stress and exerting cytoprotective effects;
5) Improving steroid responsiveness;
6) Exerting anti-inflammatory activities.

4.1. Effects of carbocysteine on mucins and ciliary functions

Goblet cell hyperplasia and metaplasia are frequently found in the airway epithelium of COPD and some more severe asthmatics patients. These events cause changes in the glycoprotein components present in the mucus that alter its viscous properties [20]. Glycosidases or glycosyltransferases (fucosidase, sialidase, fucosyltransferase, and sialyltransferase) are responsible for these changes observed in patients. The polymeric mucins MUC5AC and MUC5B are integral components of airway mucus. MUC5AC is localized in goblet cells present at the surface epithelium and in the terminal secretory ducts of submucosal glands. MUC5AC secretion, in addition to airway smooth muscle contraction, is necessary for airway hyper-responsiveness (AHR) and appears to be detrimental in acute lung injury, enhancing neutrophil trafficking and inflammation and, the severity and abundance of mucus plugging. MUC5B protein is localized in the mucous cells of submucosal glands and, to a lesser extent, in secretory cells within the surface airway epithelium of the trachea and bronchi. MUC5B is critical for mucociliary clearance and

airway defense. Muc5b-deficient mice accumulate aspirated materials in the airways and develop chronic bacterial infections, severe inflammation, and airway obstruction.

Carbocysteine can increase cilia beat in airway epithelial cells thus improving the function of the mucociliary escalator and its function to remove harmful particles, virus, and bacteria from the airway surface [21].

In models employing sulphur dioxide (SO2) to induce airway insult, carbocysteine can counteract the enhanced inflammatory cell infiltration as well as increased mucus cell numbers and MUC5AC protein expression [22]. Furthermore, it has been demonstrated the ability of carbocysteine to rebalance the altered glycosidases or glycosyltransferases activities, the MUC5AC mRNA and protein increased levels due to repeated SO2 exposure in a rat model [23] and to restore the physiological sugar composition of mucus regulating fucose and sialic acid contents on mucins [24]. Carbocysteine preserves this regulatory function on mucus composition also in pathological models where mucus viscosity alteration is promoted by Tumor Necrosis Factor (TNF) [24].

4.2 Effects of carbocysteine on viral infections

Viruses are detected in half of all COPD exacerbations and are associated with poorer clinical outcomes. Rhinovirus (RSV), respiratory syncytial virus, and influenza are the most commonly detected viruses during exacerbation. Moreover, viral pathogens may play an important role in driving COPD progression by acting as triggers for exacerbation and subsequent lung function decline [25]. In this regard, it has been demonstrated that carbocysteine pretreatment reduces RSV receptor Intercellular Adhesion Molecule 1 (ICAM-1) expression as well as a viral titer and virus RNA in vitro by using human tracheal epithelial cells [26]. Carbocysteine is also able to reduce the levels of cytokines (Interleukin (IL)-6, IL-8, IL-1) released in tracheal epithelial cells infected with human RSV [27].

Yamaya M et al have evaluated, using an *in vitro* model of the airway (human tracheal) represented by epithelial cells infected with FluA virus, the efficacy of carbocysteine as an inhibitor of this virus. Interestingly, carbocysteine can reduce viral titers, RNA levels from FluA virus, and pro-inflammatory signals including IL-6 cytokine and nuclear expression of the pro-inflammatory transcription factor Nuclear Factor Kappa B (NFkB). Moreover, it diminishes sialic acid with α2,6 linkage, a receptor for influenza virus [28]. Considering that there are currently few specific and effective antiviral strategies for patients with COPD, carbocysteine may contribute to managing viral infections.

4.3 Effects of carbocysteine on bacterial infections

The adhesion of bacteria to pharyngeal epithelial cells is the initial step in the pathogenesis of bacterial infections. Disruption of bacterial-host cell interactions with receptor antagonists or modulation of the ensuing inflammatory profile presents attractive avenues for therapeutic development. Carbocysteine can reduce the attachment of *Moraxella catarrhalis*, non typable *Haemophilus influenzae* [29,30], and *Streptococcus* (*S.*) *pneumonia* [31]. Carbocysteine is also able to induce the detachment of already adherent bacteria to epithelial cells [31]. While pro-inflammatory responses upregulate the expression of platelet-activating factor receptor (PAFR), a receptor used by *S. pneumonia* to firmly adhere to epithelial cells, carbocysteine can reduce PAFR mRNA and protein thus decreasing bacterial adherence also in a proinflammatory microenvironment [32]. Moreover, carbocysteine allows antibiotics to penetrate more easily through the hemato-bronchial barrier. The association of amoxicillin and carbocysteine leads to an enhancement in antibiotic levels in the bronchial secretion (also if it is purulent), performing a sterilizing action in a short time with significant therapeutic advantage [33].

4.4 Antioxidant and cytoprotective effects of carbocysteine

Oxidative stress induced by chronic smoke exposure is considered to be a crucial event in COPD pathogenesis [34]. Increased oxidative burden and antioxidant imbalance generated by inhaled oxidants or by endogenous sources are involved in cellular and tissue damage. For this purpose, strategies aimed at reducing oxidative burden or rising antioxidants are central in the therapy of COPD.

Different cellular models with increased oxidative stress are generated using hydrogen peroxide (H2O2), hypochlorous acid (HOCl), hydroxyl radical (•OH) or cigarette smoke extracts (CSE). Using an experimental method with •OH to enhance oxidative stress, Garavaglia ML et al demonstrated that carbocysteine exerts scavenger activity restoring physiological levels of Reactive Oxygen Species (ROS) and preventing the irreversible reduction of both glutathione (GSH) and chloride currents generated by •OH exposure [35]. Furthermore, Guizzardi F et al demonstrated that the rebalancing effects of carbocysteine on the GSH levels were dependent on the correct functionality of the Cystic Fibrosis Transmembrane Regulator (CFTR) [36]. For this reason, this efflux of GSH was not observed in diseases such as cystic fibrosis in which a correct function of CFTR is lacking.

The antioxidant activity of carbocysteine is strictly linked to cytoprotective and anti-inflammatory activities since it: a) reverts the inactivation of α1-antitrypsin; b) reduces the higher production of IL-8 due to increased intracellular •OH activity [37]. Bazzini C et al show that CSE significantly promotes cell mortality in a time and dose-dependent manner, *via* an apoptosis-independent pathway. Short-term CSE exposure induces an increase in ROS levels and a GSH intracellular concentration drop. In parallel, the expression of glutathione peroxidases 2 and 3, glutathione reductase, and glutamate-cysteine-ligase is incremented. Carbocysteine is effective in counteracting all these effects [38]. Concerning cell apoptosis, Yoshida M et al demonstrated that carbocysteine reduces the increased cell apoptosis (Caspase (CASP)3 and CASP9 activation) in tracheal epithelial cells exposed to H2O2 [39]. Hanaoka M et al showed that carbocysteine lessens lung emphysema (alveolar enlargement and parenchyma destruction) caused by cigarette smoke exposure in rats. Carbocysteine preserves lung and systemic antioxidant mechanisms and diminishes both cell apoptosis and matrix metalloproteinases (MMP)-2 and MMP-9 activity [40].

Evidence supports a pro-senescence role of increased oxidative stress. In this regard, Pace E et al showed the role played by CSE not only in decreasing cell proliferation but also in promoting a senescent functional profile of bronchial epithelial cells. Thus, nuclear expression and activity of Sirtuin1 (SIRT1) and Forkhead box O3 (FoxO3) are reduced, whereas beta-galactosidase staining increases. In CSE stimulated bronchial epithelial cells, carbocysteine reverts all these phenomena by inducing cell proliferation, SIRT1 and FoxO3 nuclear expression, and by lowering beta-galactosidase staining [41].

4.5 Effects of carbocysteine on steroid responsiveness

The inflammatory events present in COPD or more severe asthmatic patients in stable conditions are often resistant to corticosteroids [42]. Oxidative stress contributes to the low response rate to corticosteroids through the downregulation of histone deacetylase (HDAC) activity, a crucial event in the transrepression activities of corticosteroids [43]. HDACs, deacetylating histones which bind to inflammatory gene promoters, limit the access of the transcriptional machinery to these genes thus repressing their transcription [44]. CSE exposure causes marked pathological features of COPD which are insensitive to dexamethasone and are associated with downregulation of HDAC2 activity. Song Y reported that increased oxidative stress contributes to steroid insensitivity in COPD or more severe asthmatic patients [45]. These authors provide evidence supporting that, in *in vitro* and *in vivo* animal models of oxidative stress-mediated steroid insensitivity, carbocysteine restores oxidative stress levels by reducing reactive oxygen species and by

promoting GSH and superoxide dismutase (SOD) activity [46]. The increased thiol-GSH levels due to carbocysteine increase HDAC2 recruitment and suppress H4 acetylation of the IL-8 promoter, potentiating dexamethasone activity also in high oxidative stress models.

The dexamethasone insensitivity observed in the COPD rat model is improved by carbocysteine. Carbocysteine is crucial in inhibiting chronic lung inflammation (total and differential inflammatory cell counts, inflammatory cytokines release, and inflammatory cells infiltration) and ameliorating airway remodeling (thickness of airway epithelium and smooth muscle and airway fibrosis). Moreover, it can improve emphysema (emphysema index D2, level of MMP-9 in bronchoalveolar lavage fluid (BALF), and the expression of a-1 antitrypsin) and prevent impairments of lung function (PEF, Intra-tracheal Pressure (IP), and IP-slope). In in vitro model of bronchial epithelial cells, carbocysteine, differently from fluticasone propionate, can counteract CSE-mediated effects reducing oxidative stress as well as increasing HDAC2 [47]. These observations are further corroborated in a more recent paper in which it is shown that CSE decreases HDAC3 expression and, as consequence, increases acetylation processes within the cells. The acetylation improvement promotes the expression of the pro-inflammatory transcription factor p-cAMP responsive element binding protein-1 (p-CREB), IL-1 mRNA, and neutrophil chemotaxis [48]. Instead, as CSE-exposed cells are incubated with carbocysteine and beclomethasone, deacetylation processes are induced, and HDAC3 inhibits p-CREB and IL-1 mRNA expression levels as well as neutrophil chemotaxis.

4.6 Anti-inflammatory activities of carbocysteine

A key component of innate immunity and innate defense mechanisms is represented by the Toll-Like Receptor (TLR) family [49]. TLRs, predominantly expressed by monocytes/macrophages and neutrophils [49], have been also identified on lung and bronchial epithelial cells [50].

The airway epithelium is emerging as a regulator of innate immune responses to a variety of insults including cigarette smoke. Products derived from epithelial cell injury can act as ligands for TLR4 and TLR2, thus amplifying inflammatory responses within the airways. In this regard, it has been demonstrated that upon CSE stimulation, airway epithelium can release increased concentrations of IL-8 which sustain the influx of neutrophils into the airways consequently triggering innate immunity responses [50]. Neutrophils represent the predominant cell type in COPD patients. Neutrophil elastase (NE) promotes mucous cell metaplasia in chronic bronchitis and is actively involved in the protease/anti-protease imbalance, a phenomenon that leads to lung tissue destruction and emphysema [4,51]. *In vitro* and *in vivo* studies provide evidence for the anti-inflammatory activities of carbocysteine on the above-mentioned pro-inflammatory mechanisms.

5. *In vitro* and *in vivo* studies of carbocysteine effects

5.1 In vitro studies of carbocysteine effect over different cell lines

Carbocysteine counteracts some pro-inflammatory CSE-mediated effects in a human bronchial epithelial cell line (16-HBE). In CSE-stimulated bronchial epithelial cells, carbocysteine has been demonstrated to play a crucial role in the reduction of TLR4 expression and Lipopolysaccharide (LPS) binding, IL-8 mRNA, and IL-8 release due to IL-1 stimulation and neutrophil chemotactic activity [52]. Similar anti-inflammatory effects of carbocysteine are observed in nasal epithelial cells exposed to CSE [53] providing compelling evidence that carbocysteine may be considered also a promising therapeutic strategy in

chronic inflammatory nasal diseases. The effects of carbocysteine on neutrophils are also confirmed by other observations. Ishii Y et al shows that carbocysteine can reduce neutrophil chemotaxis not only by inhibiting N-formylmethionyl-leucyl-phenylalanine (fMLP)-mediated neutrophil adherence to pulmonary vascular endothelial cells but also decreasing the production of inositol 1,4,5-triphosphate (IP3) and diacylglycerol in neutrophils [54]. Other than reducing neutrophil chemotactic molecule expression/release, adhesion, and chemotaxis of neutrophils, Nogawa H et al stated that carbocysteine decreases rat neutrophils. Moreover, it shows scavenger effects on H_2O_2, HOCl, •OH and Peroxynitrite (ONOO-) thus reducing further pro-inflammatory responses (IL-8 and IL-6 release) [55]. In vitro models of distal airways (A549 cell line) cultured with or without H_2O_2 [56] provide evidence that carbocysteine counteracts H_2O_2 effects by increasing cell viability, decreasing Lactate Dehydrogenase (LDH), IL-6, and IL-8 in cell supernatants, and attenuating activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and NFkB. In the same in vitro model, Wang W et al demonstrated that carbocysteine, administered to the cells 24 hs before or after TNF stimulation, can regulate the release of IL-6 and IL-8 as well as the activation of ERK1/2 and NFkB [57].

5.2 In vivo studies of carbocysteine effect over animal models

Asti C et al by using in vivo models demonstrated the efficacy of carbocysteine administered either by oral or inhalation route to reduce airway hyper reactivity and inflammation promoted by smoke exposure [58]. In mice treated with intratracheal instillation of LPS, carbocysteine decreased neutrophil numbers by increasing the binding of apoptotic neutrophils to alveolar macrophages and promoting neutrophils phagocytosis [59]. Song Yan generated a COPD mice model by instilling LPS and cigarette smoke exposure to study carbocysteine effects. He stated that carbocysteine significantly restored the MUC5B/MUC5AC ratio, together with decreased neutrophil counts, keratocyte-derived cytokine and IL-6 levels, and TNF-α mRNA expression in these mice model. Furthermore, carbocysteine significantly improved lung function, as reflected by airway resistance and dynamic compliance [60].

6. *In vivo* studies of carbocysteine effects in COPD patients

Limited evidence demonstrates the *in vivo* anti-inflammatory or antioxidant activities of carbocysteine in COPD or asthmatic patients (Fig.2).

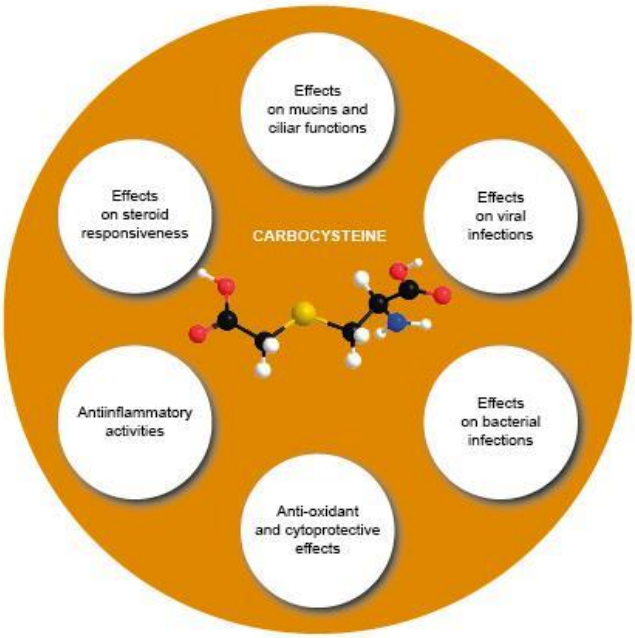

Figure 2. Overview of the various effects of carbocysteine with positive impact in COPD.

In this regard, Carpagnano GE et al measured 8-isoprostane and IL-6 levels in exhaled breath condensate of mild acute and mild stable COPD. She demonstrated that carbocysteine can decrease the high concentrations of these mediators present in patients [61]. Furthermore, a recent study provides data on short-term treatment (20 days) with or without carbocysteine to manage mild AECOPD demonstrating that the addition of carbocysteine in mild AECOPD patients improves symptoms, Forced Expiratory Volume in the 1st second (FEV1) and forced expiratory flow at 25–75% of forced vital capacity (FEF25-75) while it increases circulating sRAGE and reduces miR-21, IL-8 and fAGEs [62].

6.1 Clinical studies of carbocysteine effects in COPD

To this date, few clinical studies regarding carbocysteine effects in COPD patients are available. In the UK, before 1990, small-scale studies have investigated the effect of daily administration of carbocysteine (2,25–3g) *versus* (*vs*) placebo in chronic bronchitis. The results were rather heterogeneous in terms of FEV1 and peak flow rate but the outcome regarding more subjective parameters such as cough, dyspnea, and increased sputum clearance was consistently positive [63-66] (Fig.3 and Table 1).

Figure 3. Main clinical studies to understand Carbocysteine effects on COPD patients.

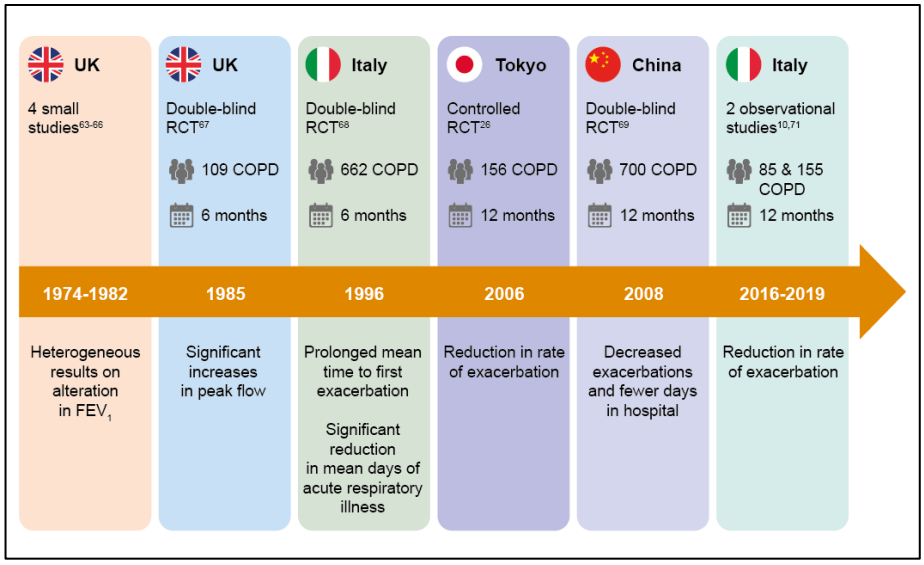

Table 1. Clinical studies on the efficacy of carbocysteine treatment in COPD patients.

Clinical Trial	Study design and enrolled patients	Therapeutic regimen	Outcome
[63-66]	Small scale UK studies on patients with chronic bronchitis	2,25–3,00 g carbocysteine daily along *vs* placebo	Heterogeneous results on alterations in FEV_1, peak flow rate, and dyspnea scores
[67]	A double-blind, parallel-group study in the UK in 109 patients with chronic bronchitis over 6 winter months	750 mg carbocysteine three times daily compared with placebo on peak flow and exacerbation rate.	No significant difference in exacerbation rate. Significant increases in peak flow from baseline in both placebo and intervention groups
[26]	Placebo-controlled RCTs in Tokyo in 156 patients with COPD over12 months	1,5 g carbocysteine daily with placebo	Significant reduction in the number of common colds and reduction in the rate of exacerbation
[68]	An Italian multicenter, prospective, double-blind RCT involving 662 outpatients with a chronic bronchitis	2,7 g SCMC-Lys once daily for 6months on COPD patients	No significant difference in baseline FEV_1 between the groups. Meantime to first exacerbation was a significantly prolonged and significant reduction in mean days of acute respiratory illness per patient.
[69]	Multicenter, randomized, double-blind, placebo-controlled, parallel-group study in China involving more than 700 COPD patients with a history of at least two COPD exacerbations within the previous 2 years	1500 mg/day carbocysteine for one year	Long-term (one year) use of carbocysteine produced a reduction in the numbers of exacerbations in patients with COPD.

			Decreased exacerbations and fewer days in the hospital. No loss of lung function and improvement in health-related quality of life.
[10]	An Italian observational and prospective study including 85 COPD out-patients with a history of at least 1 COPD exacerbation within the previous year.	2,7 g of carbocysteine daily for one year	Reduction in the exacerbation rate after 12 months of therapy, completely independent from the use of ICS. Statistically significant improvement in the quality of life assessed (decrease in SGRQ score) and the distance walked (6MWT), with a significant reduction in the BODE index. No significant improvement in lung function (FEV1, FVC, FEV1/FVC).
[71]	Observational prospective study on 155 consecutively enrolled COPD patients with a history of at least 1 COPD exacerbation within the previous year.	2,7 g of carbocysteine daily for one year	Reduction of exacerbations number at 1-year evaluation irrespective of treatment with or without ICS.

An earlier more accurate study developed in the UK with double–blind, parallel-group compared the treatment effects after administration of 750 mg of carbocysteine 3 times daily vs placebo in 109 patients with chronic bronchitis over 6 winter months. The results suggested no significant differences in the exacerbation rate and the peak flow increase from baseline in both placebo and intervention groups [67] (Fig.3 and Table 1). On the other hand, two RCTs conducted in Tokyo compared 1,5 g of daily carbocysteine with placebo in 156 COPD patients over 12 months. This study disclosed a significant reduction in the number of common colds with a lower exacerbation rate in COPD patients [26]. None of the patients received ICS or oral corticosteroids during the study. Moreover, an Italian multi-center, prospective, double-blind RCT involving 662 COPD patients reported no significant difference in baseline FEV1, but a significantly prolonged meantime until the first exacerbation episode in patients treated with 2,7g SCMC-Lys once daily for 6 months compared to placebo [68] (Fig.3 and Table 1).

The PEACE study is the first clinical trial that closely follows a reliable research study design to clarify whether COPD patients can benefit from protracted mucolytic therapy [69]. This study was classified as a multicenter (22 centers in China), randomized, double-blind, placebo-controlled, parallel-group study. To be enrolled in this study patients had to have a history of at least two COPD exacerbations within the previous 2 years and be considered clinically stable for over 4 weeks before the study. The PEACE study gathered more than 700 COPD patients that were followed for 1 year. The cumulative number of exacerbations for the whole year was 325 in the carbocysteine group and 439 in the placebo group, corresponding to 1,01 (SE 0,06) exacerbations per patient/year with carbocysteine treatment *vs* 1,35 (SE 0,06) with placebo. The risk ratio of exacerbation was 0,75 (95% CI 0,62-0,92, p =0,004). Interestingly, the number of acute exacerbations in COPD patients who received carbocysteine (1500 mg/day for a year) decreased by 24% compared to the placebo group. Moreover, carbocysteine demonstrated a better rate of prevention in patients who suffered frequent exacerbation events, but the preventive effects were found only in patients treated with carbocysteine for 6 months or more [69] (Fig.3 and Table 1).

These results may lead to the conclusion that the anti-inflammatory and antioxidant properties of carbocysteine in COPD patients require time to be effective. Thus, the longer

is the carbocysteine administration, the better are the preventive effects on recurrent exacerbation. Non-significant interaction was found between the preventive effects and COPD severity, smoking, and concomitant therapy. The advantage of carbocysteine over placebo in the prevention of an exacerbation was noteworthy even after adjustment for concomitant use of ICS. This finding has been objecting to debate particularly because it differs from the BRONCUS study [70] in which a significant reduction in exacerbation rate by N-acetylcysteine was shown only in patients without concomitant use of ICS. The authors of the PEACE study themselves provide some reasonable explanations for such differences. First of all, the response to treatment could be associated with patients' ethnicity, as PEACE study patients were Chinese. Secondly, PEACE patients were receiving ICS in a lower percentage and a smaller dose compared with BRONCUS participants. As such, the effects of carbocysteine would be more readily identified in Chinese patients with modest use of concomitant ICS, than in the BRONCUS study.

Mucolytics such as carbocysteine seem unlikely to replace ICS in the treatment of COPD but might be an important alternative where corticosteroids use is contraindicated. The study has also documented improvements in St George's Respiratory Questionnaire (SGRQ) total score and symptom score that were observed as clinically relevant. The nature and incidence of adverse events were similar to previous studies and did not present differences between carbocysteine and placebo groups for 1-year treatment protocol, confirming carbocysteine well tolerability for long-term treatments.

More recently, the observational and prospective CAPRI study included 85 COPD patients with a history of at least one COPD exacerbation within the previous year. Enrolled patients were treated with daily administration of 2,7g of SCMC-Lys and examined every three months until the end of the study (i.e., 1 year). The primary endpoint, the reduction in the exacerbation rate, was reached after 12 months of therapy, whereas the PEACE study achieved the same result in six months. Interestingly, the decrease in the exacerbation frequency was completely independent of the use of ICS, contrasting with the BRONCUS study [10]. The improvement in the quality of life was assessed as one of the secondary endpoints. The results showed an improvement in this parameter by a statistically significant decrease in SGRQ score, according to the PEACE study. The authors did not record a significant improvement in lung function (FEV1, FVC, FEV1/FVC). The BODE index was another parameter analyzed. The BODE index is the best predicting parameter (concerning FEV1) for estimating the risk of mortality in patients with COPD both from general and from respiratory causes. In this study, the BODE index showed promising results as it was significantly reduced and correlated with a significant increase of the distance walked (6MWT).

Finally, the results from CAPRI study were confirmed by Paone et al. By the enrollment of 155 COPD patients, they demonstrated that the addition of a single dose of carbocysteine lysine salt to background therapy was able to significantly reduce the 1-year exacerbation rate. Notably, as for CAPRI primary outcome, the decrease was completely independent of the use of inhaled steroids [71] (Fig.3 and Table 1).

In 2019, the Cochrane meta-analysis for long-term use of mucolytic agents established a moderate statistically significant reduction in the number of exacerbations per patient [2]. This meta-analysis included 30 placebo-controlled, randomized clinical trials (RCTs) and involved a total of 10377 patients. All trials had a randomized, double-blind, parallel-group design. Out of those 30 studies, only five included in the meta-analysis concerned carbocysteine, and only one specifically evaluated the long-term use of carbocysteine [72]. From the four studies selected only PEACE study was defined as high quality.

The analysis involved 1357 patients treated for 10,4 months (range 6-12 months) with 1500 mg carbocysteine daily dose. The results showed that long-term use of carbocysteine reduced 0,43 exacerbations per participant per year (95% CI -0.57, -0.29) and decreased the number of patients with at least one exacerbation. Moreover, the long-term use of carbocysteine in patients with COPD may improve the quality of life and it was well-tolerated.

Unfortunately, substantial heterogeneity was detected in the outcomes. This heterogeneity decreased when the analysis was limited to the population of non-Chinese ethnicity. However, the fact that different races could contribute to the heterogeneity observed is still under scrutiny and remains a controversial point.

7. Conclusions

Although carbocysteine is still considered by many to be only a mucoactive drug, it exhibits a wide range of *in vitro* and *in vivo*, anti-inflammatory and antioxidant activities studied in both animal and human models.

Carbocysteine may diminish inflammatory cell recruitment to the airways, attenuate endothelial injury and associated cough sensitivity, and act as a potent, free-radical scavenger. Taken together these actions, in parallel with its mucoregulatory function, carbocysteine has theoretical relevance to pathogenic processes in COPD and other inflammatory airways disease. Clinical studies performed in COPD patients to check the effects of carbocysteine have produced heterogeneous results in terms of several clinical outcomes, including the exacerbation rate and lung function parameters. This heterogeneity has also been seen in further studies analyzing other mucolytic agents.

To this date a trial specifically focused on the effects of carbocysteine for long-term use has not been conducted yet. Thus, the information available concerning the direct impact of carbocysteine on disease progression, survival, and efficacy in the case of simultaneous use of ICS in COPD patients is very limited.

Esposito et al and Paone et al showed carbocysteine effectiveness in reducing COPD exacerbations rate independently from corticosteroids use. Indeed, they observed a significant reduction in exacerbation number in individuals with more than 2 exacerbations at baseline.

It is important to emphasize that although those studies have some pitfalls, they are a single site, non-RCT, with a limited number of enrolled subjects; however, their observational design and the applicability of the findings to a general, real-life, clinical practice, may have overcome these shortcomings.

Other well-conducted studies exploring these debated questions are therefore strictly desirable. Currently, the use of carbocysteine in COPD remains a well-tolerated treatment with a favorable safety profile that provides symptomatic relief to many patients with mucus production. The effect on the exacerbation rate appears to be related to the duration of therapy.

This clinical efficacy can be related to its activity in several molecular mechanisms including mucin and ciliary function modulation, counteraction to oxidative stress, viral and bacterial infections, promotion of anti-inflammatory activities and improvement to steroid responsiveness.

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